Development of a Perfusion Bioreactor System for Real-Time Viability Assessment

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Islet Transplant and Encapsulation:

- **Islet transplantation** is a promising treatment for type 1 diabetes, an autoimmune disease targeting the insulin producing cells of the pancreas.
- The **TheraCyte** encapsulation device protects the transplanted **islets** (clusters of pancreas cells) from the immune system while allowing the cells access to nutrients and oxygen.
- **Oxygen consumption rate** (OCR) is an indicator of cell viability [1].

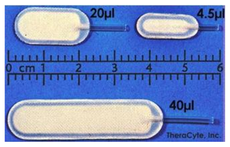


Figure 1: The TheraCyte Device for islet transplant.

Objectives:

This project was focused on the development of a device to study the effect of various oxygen and glucose environments on the oxygen consumption rate of encapsulated islets *in vitro.* The device will be used to better understand the behavior and oxygen requirements of transplanted islets *in vivo.*

1. **Develop a 3-point calibration procedure** which will enable accurate oxygen measurement from 0 to 760 mmHg.
2. **Characterize the bioreactor system**
   - Compare two bioreactor designs
   - Determine factors affecting measurement
   - Identify requirements of the next design iteration

The Perfusion Bioreactor System:

- **Fiber optic oxygen sensors** detect the partial pressure of oxygen (pO_2) in the medium. The pO_2 is measured at the inlet and outlet of the chamber.

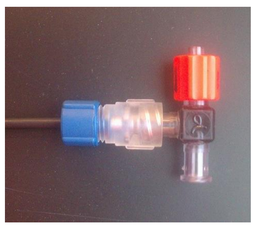


Figure 2: And oxygen sensor used to measure pO_2.
Figure 3: The perfusion bioreactor system.

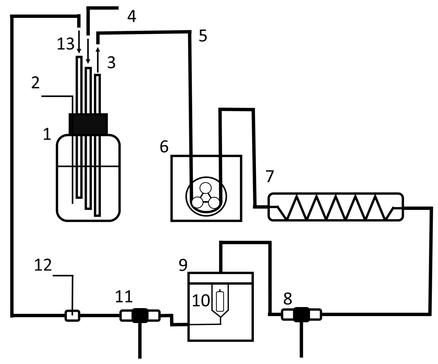
- OCR is calculated using a **mass balance equation** adapted from whole organ OCR measurement [2]:

$$OCR = \frac{Q \times (pO_{2,inlet} - pO_{2,outlet}) \times \alpha}{n}$$

Equation 1: Mass balance equation for determining OCR (fmol/min/cell) from inlet and outlet pO_2 (mmHg), flowrate (Q, mL/min), the solubility of oxygen in medium at 37°C (α=1.27 nmol/mL·mmHg), and the number of encapsulated cells (n).

The Perfusion Bioreactor System:

- The perfusion bioreactor system provides a temperature controlled and supportive environment with real-time viability assessment.



1. Medium reservoir, high glucose DMEM
2. Temperature probe
3. Medium outlet
4. Gas flow into medium
5. Masterflex tubing
6. Peristaltic pump, Instech
7. Heat exchangers
8. Fiber-optic flow through oxygen probe, arterial
9. Bioreactor
10. TheraCyte
11. Fiber-optic flow through oxygen probe, venous
12. In-line temperature probe
13. Medium return

Figure 3: A schematic of the perfusion bioreactor.

Results: A Three Point Calibration Procedure

- An empirical **second order polynomial equation** which describes the relationship between the pO_2 and the fluorescent decay time (τ) was used to generate a **calibration curve**:

$$\frac{\tau_0}{\tau} = 1 + K_1[pO_2] + K_2[pO_2]^2$$

Equation 2: The equation used to calibrate the oxygen sensors for use from 0 to 100 % O_2. τ_0 is the decay time at 0% O_2. K_1 and K_2 are constants.

Methods: To calibrate the system τ_0, τ_{21}, and τ_{100} were measured in medium bubbled with nitrogen, lab air, and oxygen respectively for each sensor.

Three Point Calibration Curves:

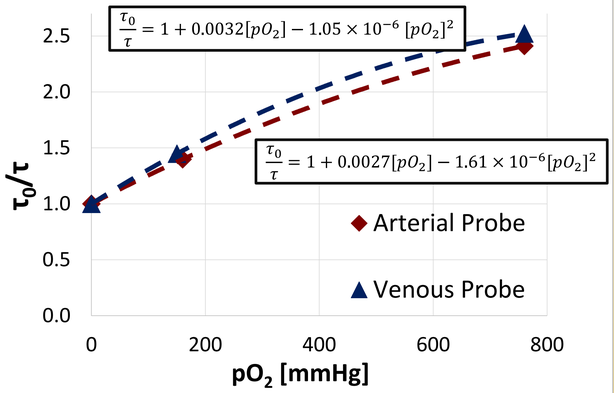


Figure 4: An example of two calibration curves.

Results: Comparison of Bioreactor Designs

Methods: βTC-3 cells were cultured, encapsulated in a TheraCyte device, and the perfusion bioreactor system was used to measure the cellular OCR. The OCR of cells from the same passage was measured in a stirred micro-chamber system.

Cylindrical Chamber Bioreactor:

- The OCR was measured to be 982 nmol/min·mgDNA in the perfusion bioreactor which differed significantly from the micro-chamber measurement of 128 nmol/min·mgDNA.
- Possible reasons for the large over estimate of OCR include oxygen diffusion from the chamber between the probes, error from the calibration due to oxygen exchange in the tubing, and possible contamination in the medium.

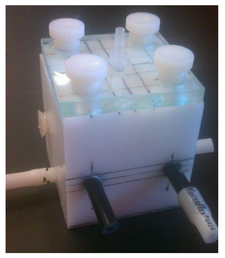


Figure 5: The cylindrical chamber bioreactor.

Flat Chamber Bioreactor:

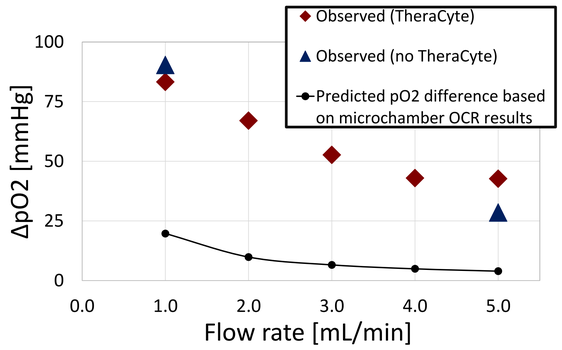


Figure 6: The observed and predicted pO_2 difference across the bioreactor. The predicted curve was calculated based on the mass balance equation (Eq. 1) and stirred micro-chamber OCR data.

- The pO_2 difference across the bioreactor decreased with flow rate, however it was not inversely proportional to the flowrate as predicted.
- The pO_2 difference was similar when measured without the TheraCyte in the chamber suggesting very little consumption and oxygen diffusion from the chamber.

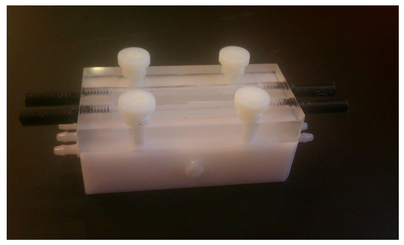


Figure 7: The flat chamber bioreactor.

Results: Factors Influencing OCR Measurement

- **Shape of chamber:** The shape of the chamber must allow medium to flow around the TheraCyte as to not limit oxygen transport into the device.
- **Temperature:** Both the solubility of oxygen in the medium and the decay time of the fluorescent dye are affected by temperature variation.
- **Oxygen diffusion:** Oxygen exchange from the medium before it reaches the oxygen sensors has a large impact on the accuracy of the calibration. Also oxygen diffusion to or from the sensors can have a significant effect on measured OCR.
- **Lower sensitivity to oxygen at high pO_2:** The oxygen sensors are less accurate and more unstable at high oxygen concentrations.
- **Flow rate** and **sensor position** do not effect oxygen measurement

Recommendations for Future Designs:

- **Membrane oxygenator:**
  - An oxygenator in the circuit before the bioreactor would ensure an accurate calibration
  - Membrane oxygenation instead of bubbling gas in medium will allow more temperature control
  - Accurate control of the oxygen level in the medium will be required for future TheraCyte experiments.
- **Oxygen impermeable bioreactor:**
  - Building the bioreactor from an oxygen impermeable material will eliminate the effect of oxygen leakage on the measured pO_2 difference.
- **Serializable:**
  - A sterile system is required for longer experiments.
- **Gas calibration:**
  - To reduce the calibration time the possibility of a gas calibration should be considered.

References:

[1] K. K. Papas, C. K. Colton, R. a Nelson, P. R. Rozak, E. S. Avgoustiniatos, W. E. Scott, G. M. Wildey, a Pisania, G. C. Weir, and B. J. Hering, "Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice.," *Am. J. Transplant*, vol. 7, no. 3, pp. 707–13, Mar. 2007.

[2] B. P. Weegman, V. a Kirchner, W. E. Scott, E. S. Avgoustiniatos, T. M. Suszynski, J. Ferrer-Fabrega, M. D. Rizzari, L. S. Kidder, R. Kandaswamy, D. E. R. Sutherland, and K. K. Papas, "Continuous real-time viability assessment of kidneys based on oxygen consumption.," Transplant. Proc., vol. 42, no. 6, pp. 2020–3, 2010.

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